

CLAIMS

We claim:

1. A method of producing cells which express a protein having factor VIII procoagulant activity comprising the sequential steps of:
 - a) obtaining cells which are solely of human origin,
 - b) contacting the cells of step a) with a vector under conditions sufficient to allow the vector to enter the cells, wherein the vector comprises a selectable marker and a sequence coding for the protein having factor VIII procoagulant activity operably linked to a promoter,
 - c) selecting the cells from step b) with a selection agent, and
 - d) isolating individual clones which express high levels of the protein having factor VIII activity from the cells obtained from step c).
2. The method of claim 1, further comprising the step
 - e) adapting the clones of step d) to growth in a plasma derived protein-free medium.
3. The method of claim 1, wherein the cells of step a) are hybrids of human lymphoma cells and 293S cells.
4. The method of claim 1, wherein the cells of step a) are hybrids of 2B8 cells (ATCC CRL-12569) and 293S cells.
5. The method of claim 1, wherein the cells of step a) are HKB11 cells (ATCC CRL-12568).
6. The method of claim 1, wherein the steps c) and d) are performed more than once.
7. The method of claim 1, wherein the sequence of step b) codes for the sequence shown in figure 1 (SEQ ID NO:1).

8. The method of claim 1, wherein the sequence of step b) codes for human factor VIII.
9. The method of claim 1, wherein the selectable marker of step b) is dhfr and the selection agent of step c) is methotrexate.
10. The method of claim 1, wherein the selectable marker of step b) is gs and the selection agent of step c) is methionine sulfoximine.
11. The method of claim 1, wherein the selectable marker of step b) is mdr and the selection agent of step c) is colchicine.
12. A method of producing a protein having factor VIII activity comprising growing the cells produced by the method of claim 1 in a growth medium, and then isolating the protein having factor VIII activity from the medium.
13. The method of claim 11 wherein the protein is human factor VIII.
14. The method of claim 11 wherein the protein has the amino acid sequence shown in figure 1 (SEQ ID NO:1)).
15. A human cell line derived from human lymphoma cells and 293S cells which expresses high levels of a protein having factor VIII activity.
16. The human cell line of claim 14, wherein the human cell line is derived from HKB11 cells (ATCC CRL-12568).
17. A human cell line derived from human lymphoma cells and 293S cells which expresses high levels of a protein having factor VIII activity when grown in plasma derived protein-free medium.
18. The cell line of claim 16, wherein the cell line is derived from HKB11 cells (ATCC CRL-12568).
19. A cell line designated 20B8 (ATCC CRL-12582).